

CLAIMS

What is claimed is:

1. A multiplex diagnostic kit comprising:

a plurality of amplification primer pairs, and a plurality of extension primers, wherein each of the plurality of amplification primer pairs has a sequence such that
 - (a) a plurality of amplicons produced from a target nucleic acid using the plurality of amplification primer pairs, respectively, includes a mutated position in a target nucleic acid;
 - (b) the plurality of amplicons is produced in a PCR reaction using the same amplification profile;and wherein each of the plurality of extension primers has a sequence such that
 - (c) each of the plurality of extension primers specifically hybridizes to each of the plurality of amplicons at the same extension temperature, respectively, such that the 3'-end of each of the extension primers corresponds to a complementary position of the mutated position, respectively; and
 - (d) selective primer extension for each of the plurality of extension primers is achieved at the same extension temperature.
2. The multiplex diagnostic kit of claim 1 further comprising a biochip to which are coupled in a plurality of distinct positions a plurality of distinct capture probes, respectively, and wherein each of the plurality of capture probes hybridizes with a portion of each of the extension primers, respectively.
3. The multiplex diagnostic kit of claim 2 wherein each of the plurality of the distinct capture probes has a unique sequence distinct from the target nucleic acid.
4. The multiplex diagnostic kit of claim 1 further comprising a DNA-dependent DNA polymerase.

5. The multiplex diagnostic kit of claim 1 wherein the plurality of amplification primer pairs has a plurality of forward primers and a plurality of backward primers, respectively, and wherein the kit includes at least two forward amplification primers having a sequence according to SEQ ID Ax and Ay, and at least two backward amplification primers having a sequence according to SEQ ID Bx and By, wherein x and y are integers between 1 and 24 and not the same.
6. The multiplex diagnostic kit of claim 1 wherein the plurality of extension primers include at least two extension primers having a sequence according to SEQ ID Cx and Cy, wherein x and y are integers between 1 and 24 and not the same.
7. The multiplex diagnostic kit of claim 1 further comprising an instruction to perform the PCR reaction and primer extension in a single tube.
8. A multiplex diagnostic kit comprising:

at least two forward amplification primers having a sequence according to SEQ ID Ax and Ay, at least two backward amplification primers having a sequence according to SEQ ID Bx and By, and at least two extension primers having a sequence according to SEQ ID Cx and Cy; and

wherein x and y are integers between 1 and 24 and not the same.
9. The multiplex diagnostic kit of claim 8 further comprising an instruction to perform a multiplex PCR using the at least two forward amplification primers and the at least two backward amplification primers using the same amplification profile.
10. The multiplex diagnostic kit of claim 9 further comprising an instruction to perform a primer extension reaction using the at least two extension primers at the same extension temperature.
11. The multiplex diagnostic kit of claim 10 further comprising an instruction to perform the multiplex PCR and the extension reaction in a single tube.
12. The multiplex diagnostic kit of claim 8 further comprising a biochip to which are coupled in a plurality of distinct positions a plurality of distinct capture probes,

respectively, and wherein each of the plurality of capture probes hybridizes with a portion of each of the extension primers, respectively.

13. The multiplex diagnostic kit of claim 12 wherein each of the plurality of the distinct capture probes has a unique sequence distinct from a target nucleic acid to which the amplification primers bind.
14. The multiplex diagnostic kit of claim 8 further comprising at least one of a reagent and an enzyme.
15. The multiplex diagnostic kit of claim 8 wherein the amplification primers and the extension primers are specific towards an HPV virus.
16. A synthetic nucleic acid having less than sixty nucleotides and comprising an HPV recognition sequence selected from the group consisting of SEQ ID Ax, SEQ ID Bx, and SEQ ID Cx, wherein X is an integer between 1 and 24, wherein no more than two nucleotides in the HPV recognition sequence are replaced by N.
17. The synthetic nucleic acid of claim 16 having SEQ ID Cx and further comprising a plurality of nucleotides at the 5'-terminus that have less than 60% homology to a target sequence to which the nucleic acid hybridizes.
18. The synthetic nucleic acid of claim 16 wherein SEQ ID Ax, SEQ ID Bx, and SEQ ID Cx are complementary to an HPV mutant.
19. The synthetic nucleic acid of claim 16 having less than 40 nucleotides.
20. The synthetic nucleic acid of claim 16 consisting of the HPV recognition sequence.